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Sustainability of Induced Systemic Resistance by Vesicular Arbuscular Mycorrhyzae *Glomus sp*. in Tomato Plants Against *Fusarium oxysporum*

Keberlanjutan Perlindungan Induksi Resistensi Sistemik oleh Mikoriza Vesikular Arbuskular Glomus sp. pada Tanaman Tomat Terhadap Fusarium oxysporum

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ABSTRACT

Induced systemic resistance (ISR) by vesicular arbuscular mycorrhizae (VAM), *Glomus* sp., has been shown effective to protect tomato plants against *Fusarium oxysporum*. Since plant-mycorrhizae interaction could happened since early growth, study of ISR sustainability need to be carried out. The experiment was done in March to June 2004 in a random block design, with grouping based on time of inoculation, and 5 replications per treatment. Representative of two experiment is presented. Tomato plantlets grown on *Glomus* sp. infested or not (control) were inoculated with *F. oxysporum* by "pin-prick" method on the basal stem, at 2, 3, 4, 5, and 6 weeks after planting (WAP). Plants were kept in the wirehouse and symptom development was observed 21 days after inoculation (DAI). The experiment showed that *Glomus* sp. infestation increased tomato plant resistance to *F. oxysporum*, and the ISR sustained until at least 6 WAP. *Glomus* sp. infestation prolonged incubation period to 7 days, lowered the percentage of infected root by 86.84%; reduced disease intensity on the root system by 86.87%; shortened lesion length by 56.25%; reduced lesion area by 30.34% and increased plant height and leaf number slightly. In this experiment, the percentage of *Glomus* sp. infestation was 69% to 80%. The shortest incubation period was 4.6 to 7.2 days in non-infested samples, and the highest percentage of infected root was 8.4-19.8%; disease intensity on root was 5.0-11.8%; lesion length was 25.0-65.9%; and lesion area was 3.9-11.7%. Those variables were 7.4-9.4 days; 1.1-2.3%; 0.7-2.0%; 8.8-25.8% and 3.1-6.4% respectively in *Glomus* sp. infested samples. With the above level of protection and sustainability, ISR by *Glomus* sp. give hope to control *Fusarium* Wilt.

Keyword: sustainability, induced resistence, tomato, MVA, Glomus sp., Fusarium

INTRODUCTION

Induced systemic resistance (ISR) had been elucidated thoroughly in cucumber and *Colletotrichum lagenarium* system (Dean and Kuc, 1986a; Kuc, 2001; Mucharromah and Kuc, 1991). In this system signal production to initiate protection occur as early as 72 hours after induction inoculation (Dean and Kuc, 1986b). Later Dalisay and Kuc (1995a) proved that the protection last for at least 4 weeks prime, then started to decrease even though the level was still higher compared to control by 6 weeks after challenge inoculation. This protection was preceded by an increase in the enzyme peroxidase, chitinase and β-1,3-glucanase activities, which also ceased before the protection level started to decrease (Dalisay and Kuc, 1995b).

In tomato plant, ISR by vesicular arbuscular mycorrhizae (VAM) *Glomus* sp., had been shown efective against *Fusarium oxysporum*. Budi (2003) showed that *Gigaspora* sp. or *Glomus* sp. infestation on tomato plant decreased disease intensity by 77,2% and 82,4% respectively. Microscopic observation had confirmed less damaged tissues on the horizontal

slices of the basal stems and roots (Murwanto *et al.*, 2001). ISR and the induction of defense responses with VAM confirm the possibility of developing VAM as an ISR agent besides its original use as a growth promoting factor (Sastrahidayat, 1997; Setiadi, 2001) with or without any effect on root disease (Bustamam, 1992). However, since in the culture practice mycorrhizae is usually applied during sowing, then it is necessary to ascertain the sustainability of the protection induced by mycorrhizae

This research was designed to elucidate how long the protection induced by *Glomus* sp. infestation applied during sowing would be able to counter *F. oxysporum* infection in tomato plant.

MATERIALS AND METHODS

Materials

This research was done at Plant Protection Laboratory and Wirehouse of Agriculture Faculty, University of Bengkulu March to June 2004. The main materials used were sterilized soil, vermicompost, *Glomus* sp isolate from PAU IPB, *F. oxysporum* isolate from Curup, river sand, small coral, alcohol 70%, aquades, tissue, PDA, KOH 10%, H_2O_2, HCl 1% dan lactofenol blue. Whereas, the main equipment used were oven (Complek Plant Care, CAT No. HD 512 R. Thermaforce L. T. D. England), polybag, germination plate (60 cm x 80 cm x 6 cm), filter paper, hand sprayer, strain of 5 mm diameter, knife, petri dish, microscope, autoclave, and inoculation equipments.

This research was done in a Randomized Block Design, with grouping according to time of inoculation, which was 2, 3, 4, 5, or 6 week after planting (WAP), and with or without *Glomus* sp. infestation. Treatments were done in five replications. Representative of two experiments is presented.

Procedures

Planting Medium Preparation. Soil was strained to remove debris and was heat sterilized for one and half hours. Soil was mixed with vermicompost by 1:3 (volume) and was put into polybag for planting medium.

Sowing and Planting. Tomato seed were sown on wetted and sterile filter paper placed on germination plate which had been layered by 1 cm thick sterile small corals, 0,5 cm thick sterile sand, and a spread of zeolit containing *Glomus* sp. The germinating tomato seeds were placed on top and then covered by another layer of 0,5 cm thick sterile sand. Twenty one days old tomato seedlings were planted into polybag and were moved to the wirehouse till the end of data collection.

Isolation of *F. oxysporum*. Inoculum was isolated from *Fusarium* sp. diseased tomato plants from Sambirejo village, Curup, Bengkulu Province, by growing basal stem and root cuts on PDA in sterile methods. Growing mycelia were identified microscopically and the selected one (Booth, 1971) was then grown into pure culture. Seven days old culture was used for inoculation.

Inoculation of *F. oxysporum*. Tomato plant was inoculated by "pin-prick" method by placing a cut of 7 days old *F. oxysporum* culture on intently injured basal stem. The culture cut was placed with mycelia facing the epidermis, and was covered for 72 hours with wet sterile cotton to protect the inoculum and promote infection. Inoculation was done according to treatment to a group of plants at one time, which were 2, 3, 4, 5 and 6 WAP.

Data Collection. Data collection was done on disease severity, which were incubation period, percentage of infected root, disease intensity on root system, and percentage of lesion length and lesion area on stem cut three weeks after inoculation. Data were analyzed with ANOVA and continued by Contrast Orthogonal Test if any different. For supporting data, phosphorus content in soil used was tested at the beginning of the experiment, and leaf phosphorus content at the end.

RESULTS AND DISCUSSION

Percentage of MVA Infestation

Observation of the root tissue (Kormanik and Grow, 1992) indicated that about 74% of *Glomus* sp. infested tomato roots has structures of MVA in form of hyphae, vesicle and or spora

(Figure 1a), which were the sign of *Glomus* sp. presence, while the un-infested did not have the structures. Both had conidia of ***F. oxysporum*** (Figure 1b).

The Effect of *Glomus* sp. Infestation on Plant Resistance to *F. oxysporum*

The plant resistance response was inferred by longer incubation period, lesser infected root, lower disease intensity on root system, and smaller percentage of lesion length and area in the stem vascular tissues.

Incubation Period. The incubation period was determined as when the lesion diameter on the infection site reached 0,5 mm. This parameter inferred how successful plant hinder infection, thus the more successful the plant, the longer incubation period would be. In this experiment infestation with *Glomus* sp. prolonged incubation period, no matter when the *F. oxysporum* inoculation was done. It could also mean that induced systemic resistance effect of *Glomus* sp. infestation last more than six weeks. This was not the case in the un-infested plants, where the incubation period became sooner by time, which indicated that tomato plant became more susceptible to *F. oxysporum* when it got older (Figure 2).

Infected Root. Infestation of *Glomus* sp. also decreased the average percentage of root infected by *F. oxysporum*, with no difference to the inoculation time, while in the un-infested plants the resistance was very limited and was decreasing over time (Table 1). Plants with no MVA infestation and inoculated 6 WAP had the highest percentage of infected root, which was averaged at 19,82 %, while the lowest was found on the *Glomus* sp. infested plants inoculated by *F. oxysporum* on 6 WAP also, which was only about 2,388%.

The total number of root on the *Glomus* sp. infested plants were about 189 pieces, while in the un-infested ones were about 107 pieces. Thus with lower number of infected root pieces, the percentage of infected root in the *Glomus* sp. infested plants became even lower. The results of this experiment showed that MVA infestation also resulted in greater root number, not only longer as had been reported before (Bustamam, 1992).

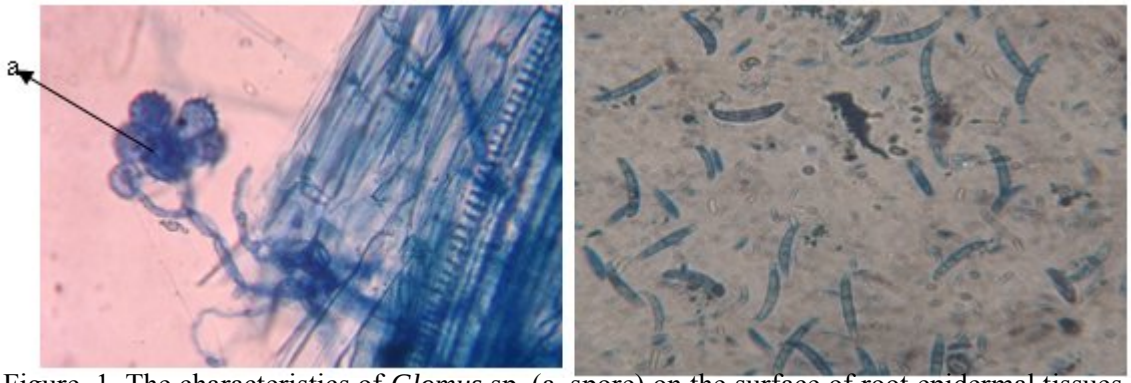

Figure. 1. The characteristics of *Glomus* sp. (a. spore) on the surface of root epidermal tissues and conidia of the infecting pathogen, *F. oxysporum* (b. macroconidia)

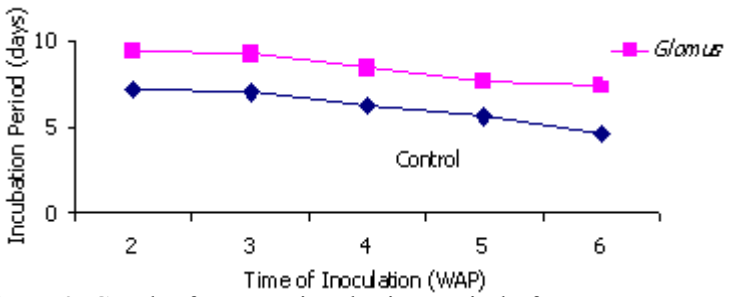

Figure 2. Graph of average incubation period of *F.oxysporum* on tomato plant infested or not with *Glomus* sp. and inoculated at 2, 3, 4, 5, and 6 WAP (Control = no *Glomus*, *Glomus* = with *Glomus* sp. infestation)

Table 1. The effect of VMA *Glomus* sp. infestation on plant resistance to *Fusarium oxysporum* inoculated at 2, 3, 4, 5 or 6 WAP

Treatment	Time of inoculation (WAP)				
	2	3	4	5	6
Percentage of Diseased Root (%)					
a. No MVA	8,44 b	10,57 b	15,07 b	16,75 b	19,82 b
b. *Glomus sp*	1,08 a	1,13 a	2,49 a	2,39 a	2,34 a
Disease Intensity on Root (%)					
a. No MVA	5,034 b	6,558 b	9,966 b	10,792 b	11,762 b
b. *Glomus sp*	0,686 a	0,586 a	1,198 a	1,542 a	1,978 a
Percentage of lesion length within stem vascular tissues (%)					
a. No MVA	25,046 b	27,736 b	41,006 b	45,676 b	65,876 b
b. *Glomus sp*	8,777 a	9,958 a	22,950 a	24,050 a	25,848 a
Percentage of lesion area within stem vascular tissues (%)					
a. No MVA	3,938 b	4,660 b	9,108 b	9,764 b	11,716 b
b. *Glomus sp*	3,052 a	3,996 a	5,994 a	6,328 a	6,384 a

Disease Intensity. The average percentage of disease intensity on root system of the tomato plants infested by *Glomus* sp. were much lower compared to the un-infested ones at all time of *F. oxysporum* inoculation (Tabel 1). This mean that the level of systemic resistance induced by *Glomus* sp. during sowing period was still efective to control Fusarium wilt even when infection occurs at 6 WAP.

The average value of disease intensity on root of the infested plants was about 1,98% whereas in the un-infested one was 11,76%. The disease intensity was increasing by the plant age in the un-infested plants, but not when plant was infested by *Glomus* sp. (Table 1).

Percentage of lesion length and area in the stem. The average percentage of lesion length and lesion area in *Glomus* sp. infested plants were lower than those of the un-infested plants (Table 1). By six weeks after planting, tomato plant infested with *Glomus* sp. became much more resistant compared to those infected at the first three weeks, particularly in terms of lesion lengths. The development of lesion length and area within the stem vascular tissues indicated that the pathogen used, *F. oxysporum* was truly a wilt pathogen, since it was able to overcome the resistance response induced within the plant tissues. However, the longer the infestation of the plant root system by *Glomus* sp., the greater the ability of the plant to prevent the disease development, thus resulted in more restricted lesion length and area (Table 1). Some of the *Glomus* sp infested plants showed recovery and left only small ex-wound signs.

Eventhough the resistance induced by MVA *Glomus* sp. against Fusarium Wilt disease in tomato plant was not absolute and still caused lesions on the vascular tissues, the difference showed in Table 1 above might be greater in the field since in this experiment inoculation was done by injuring epidermal tissues of the basal stem, thus deleting the physical barrier posed by the plant to the pathogen. This condition had united the pathogen into plant vascular tissues, which was its best habitat. Therefore the ability of the induced plants to recover and showed better protection level afterwards was very meaningful to the effectivity of *Glomus* sp. to induce systemic resistance which last for six weeks or even much longer.

Phosphorus Absorption. The results of P content analyses showed that *F. oxysporum* infection decreased P content on the leaf, thus depleting plants's energy to grow further. Therefore, the increased growth and development effect of plant by MVA infestation did not happen as much when plants were being infected by the pathogen. At the begining of the experiment P content in the soil was 6,30 ppm at soil water content of 5,48%. However, by the end of the experiment P content of the MVA un-infested plant and inoculated with *F. oxysporum* was 0,85 %, where the infested was higher (0,97 %). Under no infection, the MVA un-infested was 1,02 %, and the infested was 1,14 %. Thus, *Glomus*

sp.infestation in tomato plants induced systemic resistance and P availability for enhanced growth, as indicated by the increased available P, which is a key factor to plant growth and resistance to pathogen.

CONCLUSION

Glomus sp infestation in tomato plants at sowing effectively induced systemic resistance against *F. oxysporum* with the inhibition of infection development of 86,84% to 86,87% or very effective at the level of infected root and disease intensity percentage, and by 56,25% to 30,34% at the level of percentage lesion length and area within the plant vascular tissues. With optimal condition for successful pathogen entry in this experiment, protection level might even higher and last for 6 weeks, even longer.

In addition, ISR by *Glomus* sp. also promoted P absorption into leaves, indicating better growth even though the soil medium in this experiment was sterilized. Further research to test the effectivity in the field is needed.

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